EVALUATION OF DIAGNOSTIC CRITERIA FOR ENDEMIC NEPHROPATHY

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Abstract

Diagnosis of endemic nephropathy (EN) is based on the combination of several clinical and laboratory criteria. Despite extensive research no specific diagnostic biomarker for EN has yet been identified. The aim of the study was to evaluate the diagnostic significance of the variables previously proposed as diagnostic criteria, but also new ones. After an extended questionnaire, the clinical and laboratory examination population in EN villages was classified according to the modified WHO criteria. The urinary active form of TGF-β was measured with a bioassay using a cell line which expresses luciferase activity. In the study we used ROC analysis to examine the predictive value of the tested variables. In the study there was no difference in haemoglobin level between the study subgroups. Leucine aminopeptidase (LAP) in urine and active urinary TGF-β levels were increased in the EN diseased group when compared to other subgroups, but they did not fulfil the statistical criteria needed for differentiating a diseased form from other study subgroups. Both kidney length and parenchima thickness, alfa1-microglobulinuria, and kidney function assessed by MDRD formula were the variables that differentiated the study subgroups well. Based on our results the cut-off value of alfa1microglobulin for screening should be 23.5 mg/g creatinine instead of 15 mg/g creatinine in the present criteria, and for making a diagnosis of EN 31,5 mg/g creatinine. Persons with a positive family history for EN had a 5.8 times greater risk of developing EN when compared to a negative one.

Taken together, the abovementioned variables should be implemented in new uniform diagnostic criteria for EN.

Key words: endemic nephropathy, aristolochic acid nephropathy, diagnostic criteria, TGFbeta, alfa1-microglobulin.

Diagnosis of endemic nephropathy (EN), an environmental form of aristolochic acid nephropathy, is based on the combination of several clinical and laboratory criteria. Despite extensive research no specific diagnostic biomarker for EN has yet been identified. Currently used diagnostic criteria in different EN centres involve different combinations of parameters and various cut-off values and many of them are not in agreement with the proposed international guidelines [1–7].

The aim of the study was to evaluate the diagnostic significance of the variables previously proposed as diagnostic criteria, but also new ones.

Subjects and Methods

All inhabitants older than 18 years of age in both EN and control villages were invited to participate and were examined on a door-to-door basis. After an epidemiological questionnaire, medical history taking, and clinical and laboratory examinations, the EN population was classified according to the modified WHO criteria as "diseased", "suspected of having EN", "at risk for EN", and "others", who did not fulfil the criteria and were unrelated to EN [8].

Laboratory analyses involved determination of haemoglobin, serum creatinine, urinary α1-microglobulin, creatinine, active TGF-β and leucine aminopeptidase (LAP) from untimed spot urine sample. Haemoglobin was determined using optical fluid cytometry with the electronic counter, serum creatinine using the continuous photometric method with alkaline Picrat, and urinary α1-microglobulin by an imunonephelometric assay. The urinary active form of TGF-beta was measured with a bioassay using a cell line which expresses luciferase activity in response to active TGF-beta in a dose-dependent manner. Values were expressed as medians and 25th–75th percentiles in mg/g creatinine. Urinary LAP enzymatic activity was measured with a spectrofluorometric assay after dilution of the samples with the buffer. Results were expressed as micromoles of 7-amido-4-methyl-coumarin produced per millimole of urinary creatinine. Kidney function was assessed by estimation of the glomerular filtration rate (eGFR) using the abbreviated MDRD formula.

Kidney ultrasound was performed using an LG ultrasound machine with a sector probe of 3.5 MHz. Both kidney length and parenchima thickness were measured in sections visually estimated to represent the largest diameter.

In the study ROC analysis was used to examine the predictive value of the tested variables.

Results

In the present cross-sectional study 1140 adult persons were enrolled, 764 from EN and 376 from control villages. After an extended questionnaire and clinical and laboratory examinations, the population in EN villages was classified according to the modified WHO criteria as "diseased" (N = 35), "suspected of having EN" (N = 70), those "at risk for EN" (N = 212) and "others" (N = 447).

In the study, there was no difference in haemoglobin level between the study subgroups (P = 0.449). Even though leucine aminopeptidase (LAP) in urine was significantly higher in "diseased" when compared to other study subgorups (all P < 0.05), it did not fulfill the statistical criteria needed for differentiating diseased form other study subgroups. Moreover, active urinary TGF-beta levels were increased in the EN diseased group when compared to other subgroups. However, this increase did not meet the criteria of statistical significance when the different groups were compared. In the present study the assesment of kidney function by MDRD formula as well as both kidney length and parenchima thickness were the parameters that showed statistical significance in differentiating the study subgroups (all P < 0.05). In the present study alfa1microglobulin in urine was the best biomarker in screening and diagnosing EN. Based on our results the cut-off value of alfa1microglobulin for screening should be 23.5 mg/g creatinine instead of the 15 mg/g creatinine in the present criteria, and for making a diagnosis of EN 31.5 mg/g creatinine.

In the study persons with a positive family history for EN had a 5.8 times greater risk of developing EN when compared to a negative one.

Discussion

Diagnostic criteria for EN were described more than 50 years ago. Currently used criteria from EN centres involve different combinations of parameters, with various cut-off values and many of them are not in agreement with the proposed international guidelines.

Anaemia, characteristic of a chronic kidney disease, has not shown to be a good marker in differentiating WHO subgruops in the study. Similar results were reported by the Serbian EN centre using another diagnostic criteria for EN [4]. Therefore haemoglobin should not be

used as a diagnostic criterion for EN. Although the early phase of EN is characterized by proximal tubule damage, the proximal tubule enzyme LAP has not shown to be a good marker in differentiating the study subgroups. Again, Djukanovic et al. reported similar results using a different proximal tubule enzyme, urine alkaline phosphatase. The progression of EN, especially in the advanced stage of the disease, is characterized by interstital renal fibrosis and TGF-β in renal fibrosis plays a central role [9]. Several studies have shown no correlation between urinary TGF-β and histological findings of renal fibrosis [10–11]. In this cohort study, active TGF-beta levels were increased in the EN diseased group when compared to other subgroups. However, this increase did not meet the criteria of statistical significance when the different groups were compared. Further studies are needed in order to determine if combined with other urinary markers (microproteins), this cytokine could be helpful in discriminating patient subpopulations.

Both kidney length and parenchima thickness, alfa1microglobulinuria, and kidney funcion assessed by MDRD formula were the variables that differentiated the study subgroups well. Moreover, subjects with a positive family history of EN, i.e. the WHO subgroup of those at risk, as well as those with α1mCR greater than 23.5 mg/g creatinine who in our study partly represented persons in the WHO subgroup suspected of having EN, are persons at greater risk of developing EN and therefore should be regularly followed up by their general practitioners and nephrologists. The abovementioned variables have been implemented in recently published uniform diagnostic criteria for EN [12].

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Р е з и м е

**ЕВАЛУАЦИЈА НА ДИЈАГНОСТИЧКИТЕ
КРИТЕРИУМИ ЗА ЕНДЕМСКА
НЕФРОПАТИЈА**

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        Дијагнозата на ендемска нефропатија (ЕН)
е заснована на комбинација на неколку кли-
нички и лабораториски критериуми. И покрај
опширното истражување, сѐ уште не е иденти-
фикуван специфичен дијагностички биомаркер
за ЕН. Целта на студијата беше да се оцени ди-
јагностичкото значење на претходно предло-
жените варијабли како дијагностички критери-
уми, но, исто така, и на нови. По спроведува-
њето опширен прашалник, клиничките и лабо-
раториските испитувања на населението во се-
лата со ЕН беа класифицирани според моди-
фицираните критериуми на СЗО. Уринарната
активна форма на TGF-β се мереше со биоасеј,
со користење клеточна линија, која изразува
активност на луциферазата. Во студијата корис-
тевме анализа РОЦ за да се испита предви-
дувачката вредност на тестираните варијабли.
Во студијата немаше разлика во нивоата на хе-
моглобинот меѓу подгрупите на студијата. Ни-
вото на леуцин аминопептидаза (ЛАП) во ури-
ната и нивоата на активен уринарен TGF-β беа
зголемени во групите на заболените со ЕН во
споредба со другите подгрупи, но тие не ги испол-
ниле статистичките критериуми потребни за
диференцијација на заболената форма од други
подгрупи на студијата. И должината на бубре-
зите и дебелината на паренхимот, alfa1-микро-
глобулинуријата и функцијата на бубрезите оце-
нети со формулата на MDRD беа варијаблите
кои добро ги диференцираа подгрупите на сту-
дијата. Врз основа на нашите резултати, cut-off
вредноста на alfa1-микроглобулин за скрининг
треба да биде 23,5 mg/g креатинин наместо 15
mg/g креатинин по сегашните критериуми, и за
дијагностицирање на ЕН 31,5 mg/g креатинин.
Лицата со позитивната фамилијарна историја за
ЕН имаа 5,8 пати поголем ризик од развој на ЕН
во споредба со негативната.
        Сѐ заедно, горенаведените варијабли треба
да бидат имплементирани во новите униформи-
рани дијагностички критериуми за ЕН.

Клучни зборови: ендемска нефропатија, нефропа-
тија од аристолохична киселина, дијагностички кри-
териуми, TGF-beta, alfa1-микроглобулин.